Extent and origin of resistance to antituberculosis drugs in the Netherlands, 1993 to 2011

C Ruesen (carolienruesen@gmail.com)[1], A B van Gageldonk-Lafeber[1], G de Vries[1,2], C G Erkens[2], J van Rest[1,2], H Korthals Altes[1], H de Neeling[3], M Kamst[3], D van Soolingen[3]

1. Epidemiology and Surveillance, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
2. KNCV Tuberculosis Foundation, The Hague, the Netherlands
3. National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Citation style for this article:
C, van Gageldonk-Lafeber AB, de Vries G, Erkens CG, van Rest J, Korthals Altes H, de Neeling H, Kamst M, van Soolingen D. Extent and origin of resistance to antituberculosis drugs in the Netherlands, 1993 to 2011. Euro Surveill. 2014;19(11):pii=20738. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20738

Article submitted on 28 January 2013/ published on 20 March 2014

The elimination of tuberculosis (TB) is threatened by an apparent increase in the level of resistance in *Mycobacterium tuberculosis*. In the Netherlands, where the majority of TB patients are migrants, resistance may also be increasing. We conducted a retrospective study, using 18,294 *M. tuberculosis* isolates from TB cases notified between 1993 and 2011. We investigated the trends in antituberculosis drug resistance, focusing on the country of birth of the patients and whether resistance had developed during treatment or was the result of transmission of resistant *M. tuberculosis* strains. For both scenarios, we determined whether this had happened in or outside the Netherlands. Antituberculosis drug resistance was found in 13% of all cases analysed and showed an increasing trend among patients who had been born in the Netherlands ($p<0.001$) and a decreasing trend among foreign-born ($p=0.02$) over the study period. Since 2005, the proportion of *M. tuberculosis* resistant strains among all strains tested has increased in both groups ($p=0.03$ and $p=0.01$, respectively). Overall, we found a significantly increasing trend when excluding streptomycin resistance ($p<0.001$). The trend was most markedly increased for isoniazid resistance ($p=0.01$). Although resistance was mainly due to transmission of resistant strains, mostly outside the Netherlands or before 1993 (when DNA fingerprinting was not systematically performed), in some cases (n=45), resistance was acquired in the Netherlands. We conclude that antituberculosis drug resistance is increasing in the Netherlands, mostly related to migration from high TB-incidence countries, but also to domestic acquisition.

Introduction

Resistance to antituberculosis drugs is emerging in several areas worldwide. In eastern Europe and central Asia, hotspots of multidrug-resistant tuberculosis (MDR-TB) are present, with nearly a third of the new and three quarters of previously treated TB cases diagnosed as having MDR-TB in some countries [1]. This is of great concern, considering the limited drug options to safely and effectively treat these resistant forms of TB. Distinguishing between transmission of a resistant *M. tuberculosis* strain and development of resistance during treatment has important consequences for TB control programmes [2].

The elimination of TB (defined as less than one case per million population) – a World Health Organization (WHO) target for 2050 [3] – is threatened by an apparent increase in multidrug resistance worldwide [1,4,5]. Global trends, however, are hard to interpret as a result of incomplete coverage of surveillance data. In many regions in Sub-Saharan Africa, and also in central and eastern Europe and India, drug resistance surveillance data are lacking, mainly as a result of inadequate laboratory infrastructure [1].

In the Netherlands, a low TB-incidence country with approximately 1,000 new registered TB cases annually and an incidence of 6.0 per 100,000 population in 2011 [6], nationwide surveillance of TB has been in place since 1993. Until recently, all local mycobacteriology laboratories routinely sent their *M. tuberculosis* complex isolates to the WHO-accredited National Reference Laboratory at the National Institute for Public Health and the Environment (RIVM) for identification, drug susceptibility testing (DST) and molecular typing. After 2011, some local laboratories started to screen for resistance against first-line drugs themselves, but when resistance is diagnosed, the results are confirmed at the National Reference Laboratory, where DST is broadened to other drugs. A DNA fingerprint of each *M. tuberculosis* isolate is produced, to guide investigation of epidemiological links between TB cases.

In the mid-1990s, a descriptive study showed that the majority of TB cases with resistant strains in the Netherlands were migrants [7]. Recent increases in the proportion of migrants among TB patients in the Netherlands [6], in the prevalence of drug resistance

in many of the migrants' country of origin [8] as well as changes in the composition of the migrant population might have influenced the resistance situation in the Netherlands over the last couple of years [6,9]. To improve our understanding of the extent and origin of *M. tuberculosis* resistance in the Netherlands, we conducted a retrospective study of all TB cases notified between 1993 and 2011. We investigated the trends in resistance to antituberculosis drugs in this period, in relation to the country of origin of the patients. In addition, we assessed the extent to which drug resistance was due to transmission of resistant strains or was possibly acquired during previous treatment. For both scenarios, we determined whether this had occurred in or outside the Netherlands.

Methods

Data sources and study population

Data were obtained from three sources and matched on the basis of postal code, date of birth and sex. The resulting data set consisted of anonymous data. We used data from three sources: firstly, the Netherlands Tuberculosis Register after approval by the registry committee. These data, systematically collected at Municipal Health Services, include information on patient characteristics, treatment history, case finding and treatment outcome. Secondly, data from the National Reference Laboratory were used, which contain information on drug susceptibility and DNA profiles of the bacterial isolates. Between 1993 and 2009, nationwide fingerprinting of *M. tuberculosis* isolates using IS*6110* restriction fragment length polymorphism (RFLP) typing was performed at the National Reference Laboratory; after 2009, RFLP typing was replaced by variable number tandem repeat (VNTR) typing [10,11]. Thirdly, we received the results of epidemiological investigation of clustered cases, which is routinely carried out by TB public health nurses in the Netherlands.

All notified *M. tuberculosis* culture-positive cases between 1993 and 2011 were included in the study. Isolates with missing DST results, including those tested in local laboratories, which participate in quality control programmes, were considered susceptible. If patients had multiple isolates, only isolates of *M. tuberculosis* strains with different DNA fingerprints were included in the database: subsequent isolates representing the same *M. tuberculosis* strain were excluded.

Drug resistance: trends and origin

Standard DST for the following first-line drugs was performed for each isolate: isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide. Susceptibility to the following second-line drugs was only assessed if the isolate was resistant to isoniazid and/or rifampicin: amikacin, capreomycin, ciprofloxacin, clarithromycin, clofazimine, cycloserine, kanamycin, linezolid, moxifloxacin, ofloxacin, protionamide and rifabutin. Until 2004, the absolute concentration method was the standard DST method used [12], but this was replaced thereafter by the mycobacteria growth indicator tube (MGIT) assay [13]. Patients were classified according to the DST result as having drug-susceptible isolates if the causative bacteria were sensitive to the first-line drugs tested or as having drug-resistant isolates if resistance to at least one drug was detected.

Trends in resistance were analysed for the study period. Extensively drug-resistant TB was only detected rarely and involved in total three cases in 2009 to 2011 [6]. Drug resistance rates (percentage of resistant isolates among all isolates tested for drug susceptibility) were described for those born in the Netherlands and those who were born abroad.

A distinction was made between primary drug resistance (PDR), i.e. drug resistance in new TB cases and acquired drug resistance (ADR), i.e. drug resistance in previously treated TB cases. For foreign-born ADR patients, we compared the year of entry into the Netherlands with the year of previous TB treatment to assess whether resistance had been acquired in the Netherlands or abroad. ADR patients born in the Netherlands were considered to have acquired resistance in the Netherlands.

Transmission of drug-resistant TB

For PDR cases, we assessed where transmission of the resistant strain most probably occurred, based on the DNA fingerprinting of *M. tuberculosis* isolates and the subsequent results of cluster investigation.

Clusters were defined as groups of patients having isolates with identical RFLP or VNTR patterns or, if strains had fewer than five IS*6110* copies, identical polymorphic GC-rich sequence RFLP patterns [14]. During 2004 to 2008, within the framework of a nationwide evaluation following the introduction of VNTR typing, both RFLP and VNTR typing were performed for all isolates and strains could thus belong to both an RFLP and a VNTR cluster [15]. The agreement in clustering between both methods was about 80%. In order to prevent strains being part of two different clusters, we used the RFLP patterns to cluster isolates from before 2009 and VNTR patterns to cluster strains isolated in 2009 or thereafter. Cases were divided into those whose *M. tuberculosis* strain had a unique DNA fingerprint and those with a clustering fingerprint. The first case in each cluster, based on the diagnosis date, was classified as unique. After matching the Netherlands Tuberculosis Register data with data from the National Reference Laboratory, a number of clusters were broken up as a result of a 15% mismatch, which occurred due to incorrect or incomplete data on identifying variables (e.g. country of birth, sex, postal code) that link the cases. Cases for whom the data could not be matched were excluded from the analysis. This resulted in the formation of 'clusters' with only one case left, which were excluded from further analysis.

Table 1

Determinants of resistance to at least one antituberculosis drug in patients with culture-positive tuberculosis in the Netherlands, 1993–2011

Characteristic	Isolates with DST results n=14,820	All isolates n=14,959	Resistant isolates n=1,890	Susceptible isolates n=12,930	Crude OR (95% CI)	Adjusted OR[b] (95% CI)
	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]		
Male sex	8,857 (60)	8,941 (60)	1,106 (58)	7,751 (60)	0.94 (0.86–1.04)	1.02 (0.88–1.18)
Mean age in years (SD)	41 (20)	41 (20)	33 (15)	42 (20)	0.98 (0.97–0.98)[c]	0.98 (0.97–0.98)[c]
Living in an urban area	5,150 (35)	5,218 (35)	653 (35)	4,497 (35)	0.99 (0.89–1.10)	1.09 (0.94–1.28)
Foreign-born	10,165 (69)	10,257 (69)	1,590 (84)	8,575 (66)	2.69 (2.37–3.06)[c]	1.92 (1.55–2.38)[c]
Median number of years in the Netherlands before diagnosis (IQR)[d]	5 (1–14)	5 (1–14)	3 (1–9)	5 (1–15)	0.97 (0.97–0.98)[c]	0.99 (0.97–1.00)
BCG vaccination[e]	4,124 (50)	4,182 (50)	667 (67)	3,457 (48)	2.20 (1.92–2.53)[c]	1.06 (0.87–1.28)
Belonging to a risk group	9,265 (63)	9,335 (62)	1,390 (74)	7,875 (61)	1.78 (1.60–1.99)[c]	1.15 (0.96–1.37)
Previous TB treatment[e]	533 (4)	539 (4)	122 (8)	411 (4)	2.19 (1.78–2.70)[c]	2.31 (1.71–3.13)[c]
Median number of years between diagnosis and previous treatment (IQR)[f]	21 (4–50)	21 (5–50)	5 (2–16)	26 (6–52)	0.96 (0.95–0.97)[c]	0.98 (0.96–1.00)
Having pulmonary TB or pulmonary and extrapulmonary TB	10,015 (68)	10,112 (68)	1,243 (66)	8,772 (68)	0.91 (0.82–1.01)	0.92 (0.79–1.07)

BCG: Bacillus Calmette–Guérin; CI: confidence interval; DST: drug susceptibility testing; IQR: interquartile range; OR, odds ratio; SD: standard deviation; TB: tuberculosis.

[a] Data are presented as number (percentage), unless indicated otherwise.

[b] OR adjusted for sex, age, living in an urban area (Amsterdam, Rotterdam, The Hague, Utrecht), being born in the Netherlands/foreign born, being vaccinated with BCG, belonging to a risk group (i.e. a group with high risk of exposure to TB, such as drug users, asylum seekers, illegal residents), had previous treatment and had pulmonary TB.

[c] P<0.05.

[d] Calculated for foreign-born patients.

[e] Data were missing for BCG vaccination (888 resistant isolates; 5,648 susceptible isolates) and previous TB treatment (268 resistant isolates; 1,464 susceptible isolates).

[f] Calculated for previously treated patients.

PDR cases were classified into three groups according to a classification model previously described [16]: (i) PDR cases with an *M. tuberculosis* strain that had a unique DNA fingerprint, as well as clustered cases without a potential source case (i.e. without a preceding pulmonary TB case in the cluster) were considered infected abroad or before 1993; (ii) clustered PDR cases with a potential source case, but without a confirmed or likely epidemiological link to a previous case in the cluster were considered possibly infected in the Netherlands; and (iii) clustered PDR cases with a potential source case and a confirmed or likely epidemiological link with a previous case in the cluster were considered definitely infected in the Netherlands. An epidemiological link was considered 'confirmed' when a social contact with another patient in the cluster had been documented in an interview with the respective patients or 'likely' if the patients visited the same place at the same time (without being aware of each other's presence) [17].

Statistical analysis

We used logistic regression analyses to assess the statistical significance of trends in resistance, with antituberculosis drug resistance as dichotomous outcome variable and year of diagnosis as continuous independent variable. A trend was considered significant if the regression coefficient for year of diagnosis differed significantly from 0. A positive coefficient indicated an increasing trend and a negative coefficient indicated a decreasing trend. We calculated 95% confidence intervals (CIs) for drug resistance percentages over time, assuming the number of resistant samples was normally distributed.

Univariate and multivariate logistic regression analyses were used to examine which determinants were associated with resistance (dependent variables). Determinants evaluated were demographic variables – i.e. sex, age, living in an urban area (in one of the four largest cities in the Netherlands (Amsterdam, Rotterdam, The Hague, Utrecht), being foreign-born, length of time of residence in the Netherlands and belonging to a risk group (i.e. a group with a high risk of exposure to TB, such as drug users, asylum seekers, illegal residents), Bacillus Calmette–Guérin (BCG) vaccination, previous treatment history and having pulmonary TB.

Multivariate logistic regression was used to adjust for possible confounders. Variables with p<0.05 in the univariate analysis, as well as variables that were expected to be related to the outcome measure were used. In addition, differences between ADR and PDR cases were analysed using multivariate logistic regression with type of drug resistance (ADR/PDR) as dichotomous dependent variable and drug-specific resistance, age and being foreign-born as covariates. Crude and adjusted odds ratios (ORs) are presented with 95% CIs. All statistical analyses were performed using SPSS version 19. A significance level of 0.05 was used throughout.

Results

During 1993 to 2011, 18,294 isolates were collected from 18,274 notified TB cases. A total of 15,601 isolates (85%) could be matched with the Netherlands Tuberculosis Register data, of which 14,959 (96%) were *M. tuberculosis* cultures. A total of 14,820 of these isolates (99%) had DST results and 1,890 (13%) of these strains showed resistance to at least one antituberculosis drug. Resistance was found in 1,500 (12%) of 12,678 new cases and 122 (23%) of all 539 previously treated patients. Resistance to isoniazid and streptomycin was most common, while rifampicin, ethambutol and pyrazinamide resistance and MDR-TB (defined as resistance to at least isoniazid and rifampicin) were less frequently observed (data not shown). The mean age of *M. tuberculosis*-positive TB cases was 41 years (SD: 20). Of the 14,959 *M. tuberculosis* isolates, 8,941 (60%) were from cases who were male; 5,218 (35%) lived in an urban area; 10,257 (69%) were foreign-born and 10,112 (68%) had pulmonary TB (including those with pulmonary TB and extrapulmonary TB). Of 8,376 patients with data on vaccination and resistance status, 4,182 (50%) were BCG vaccinated (Table 1).

Trends in drug resistance

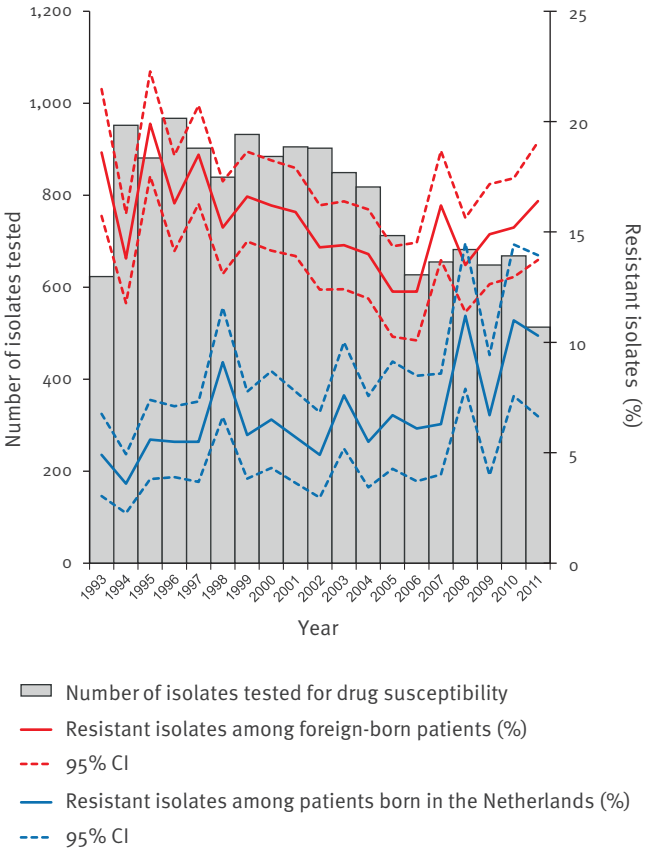
The resistance rate was considerably higher in foreign-born TB patients than in patients born in the Netherlands (16% vs 6%, p<0.001). Among those who were foreign-born, trend analysis showed a slightly decreasing trend in the proportion of resistant isolates during 1993 to 2005 (p value for trend ($p_{trend)}$= 0.01), followed by a significantly increasing trend until 2011 (p_{trend} = 0.01, Figure 1). However, the 95% CIs for 2005 and 2011 overlap slightly (10.3–14.3 and 13.7–19.1, respectively). Among patients born in the Netherlands, resistance increased from 5% (95% CI: 3.0–6.8) in 1993 to 10% (95% CI: 6.8–13.9) in 2011 (p_{trend} <0.001) (Figure 1). For the total population, the proportion of isolates resistant to any TB drug fluctuated around 12% until 2005, but then increased significantly from 10.7% (95% CI: 9.1–12.3) in 2005 to 15% (95% CI: 12.6–17.0) in 2011 (p_{trend} <0.001) (data not shown).

We found a significantly increasing trend for drug resistance, when excluding streptomycin resistance, from 7.1% (95% CI: 5.7–8.5) in 1993 to 11.1% (95% CI: 9.2–13.0) in 2011 (Figure 2, p_{trend} <0.001). Streptomycin has not been used in the Netherlands since 1996, when new TB treatment guidelines were issued, and resistance to it decreased from 5.8% (95% CI: 4.5–7.1) in 1993 to 3.7% in 2011 (95% CI: 2.5–4.9) (p_{trend} <0.001). In particular, the percentage of isolates with isoniazid resistance increased significantly from 2.9% (95% CI: 2.0–3.8) in 1993 to 5.7% in 2011 (95% CI: 4.3–7.1) (Figure 2, p_{trend} = 0.01). The same applied to MDR-TB: from 1.1% (95% CI: 0.5–1.7) in 1993 to 2.5% (95% CI: 1.5–3.5) in 2011 (Figure 2, p_{trend} <0.001).

When analysing cases by country of birth, and excluding streptomycin resistance, resistance increased from 2.3% (95% CI: 1.0–3.6) in 1993 to 8.1% (95% CI: 4.8–15.4) in 2011 among cases born in the Netherlands and increased from 10.6% (95% CI: 8.3–12.9) in 1993 to 12.2% (95% CI: 9.8–21.8) in 2011 among foreign-born cases. Isoniazid resistance increased from 0.4% (95% CI: 0.0–0.9) in 1993 to 4.4% (95% CI: 1.9–11.1) in 2011 among cases born in the Netherlands and increased

Figure 1

Trend in the proportion of *Mycobacterium tuberculosis* isolates with resistance to at least one antituberculosis drug in the Netherlands, for patients born in the Netherlands and foreign-born patients, 1993–2011

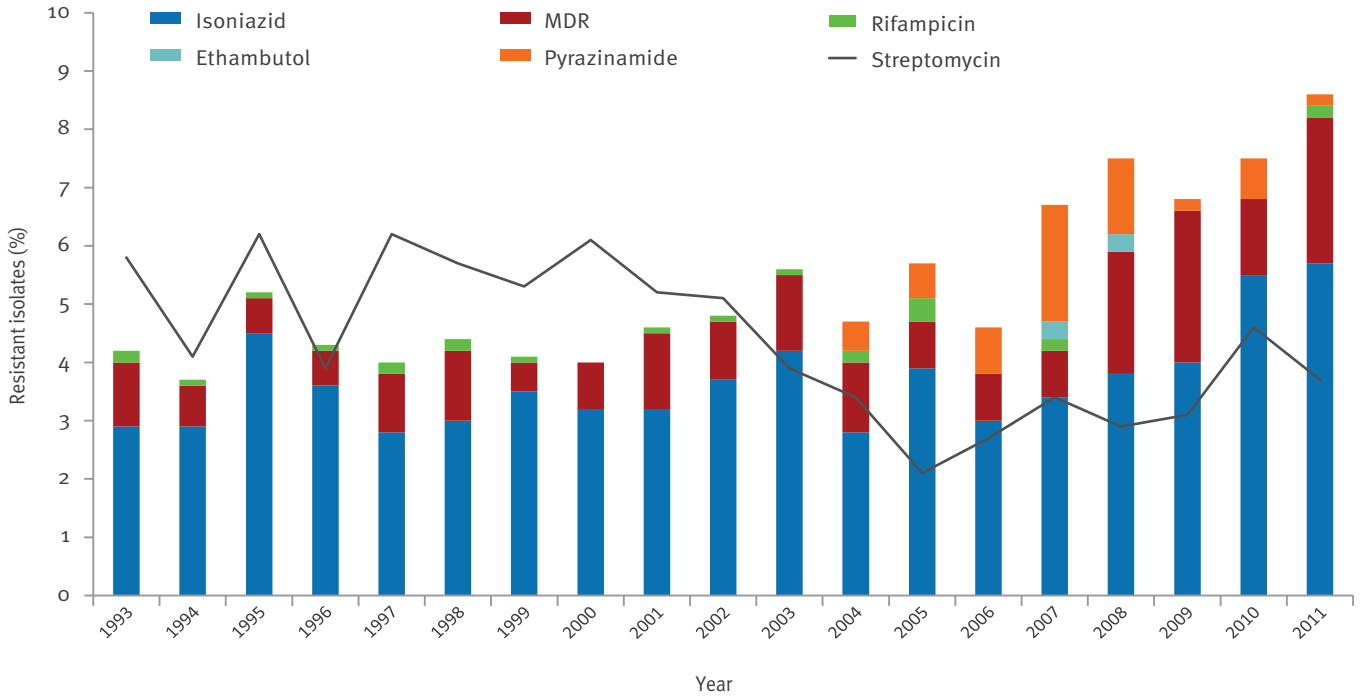


CI: confidence interval.

Data from the National Reference Laboratory at the National Institute for Public Health and the Environment (RIVM). The number of isolates tested for drug susceptibility was lower in 2011 because local laboratories started to test for drug susceptibility as well. Not all isolates were sent to the National Reference Laboratory any more.

Figure 2

Trends in the proportion of *Mycobacterium tuberculosis* isolates resistant to first-line antituberculosis drugs, the Netherlands, 1993–2011



MDR: multidrug resistance, defined as resistance to at least isoniazid and rifampicin.

Percentages calculated as the number of resistant isolates divided by the total number of isolates tested for drug susceptibility. Resistance to isoniazid and streptomycin is not shown (only mono-resistance and MDR).

from 4.7% (95% CI: 3.1–6.3) in 1993 to 6.1% (95% CI: 4.4–16.0) in 2011 among those who were foreign-born.

Origin of drug resistance

Multivariate analyses showed that younger age, being foreign-born and previous TB treatment were independently related to resistance, while sex, living in an urban area, BCG vaccination, belonging to a risk group and having pulmonary TB were unrelated (Table 1). The significant univariate association between BCG vaccination and resistance may be explained by the fact that patients who were foreign-born were more likely to be vaccinated than those born in the Netherlands. Drug-resistant isolates from foreign-born cases more often expressed rifampicin resistance and MDR than drug-resistant isolates from cases born in the Netherlands (Table 2).

For 1,622 (86%) of all 1,890 patients with drug-resistant isolates, information on previous TB treatment was available. Of these, 122 (8%) had been treated previously and 1,500 (92%) had not been treated before. Consequently, 8% of all resistant cases for whom information on previous treatment was available were classified as ADR and 92% as PDR. This corresponds to 0.8% and 10% of all 14,959 TB cases analysed, respectively. In a multivariate analysis, we found that rifampicin resistance and MDR-TB were more associated with ADR than PDR. We also found that ADR patients were older than PDR patients (Table 3).

The percentage of ADR cases was not different between patients born in the Netherlands and those who were foreign-born (Table 2). Time since previous treatment was much longer in ADR cases born in the Netherlands (mean: 22 years; SD: 19) than in foreign-born patients (mean 7 years; SD: 9); $p<0.001$). Two of 16 ADR patients born in the Netherlands were second-generation migrants as at least one parent had been born abroad. For nine ADR patients born in the Netherlands, the parents' country of birth was not registered. Of 92 foreign-born ADR patients with known date of entry into the Netherlands, 49 had previously been treated before entry and were thus considered to have acquired resistance abroad. A total of 29 foreign-born ADR patients had previously been treated after entry into the Netherlands and most probably acquired resistance or additional resistance in the Netherlands. For 14 foreign-born ADR patients, it was unknown where they acquired resistance, as the year of previous treatment coincided with the year of entry.

Transmission of drug-resistant TB

Of all 14,959 isolates, 14,913 (99.7%) DNA fingerprints were generated. Due to the 15% mismatch with Netherlands Tuberculosis Register data, 454 clusters

Table 2

Comparison of *Mycobacterium tuberculosis* isolates with resistance to at least one antituberculosis drug from tuberculosis patients born in and outside the Netherlands, 1993– 2011

Characteristic	Foreign-born n=1,590	Born in the Netherlands n=300	Crude odds ratio (95% CI)
	n (%)[a]	n (%)[a]	
Drug resistance			
Isoniazid	955 (60)	171 (57)	1.14 (0.89–1.46)
Rifampicin	183 (12)	16 (5)	2.31 (1.37–3.92)[b]
Streptomycin	1,025 (65)	176 (59)	1.28 (1.00–1.65)
Ethambutol	66 (4)	6 (2)	2.36 (1.00–5.58)
Pyrazinamide	61 (4)	19 (6)	0.64 (0.36–1.12)
Multidrug resistance (at least isoniazid and rifampicin)	156 (10)	11 (4)	2.86 (1.53–5.34)[b]
Other			
Previously treated patients with acquired drug resistance[c]	106 (8)	16 (6)	1.38 (0.80–2.37)
Mean number of years between diagnosis and previous treatment (SD)[d]	7 (9)	22 (19)	0.93 (0.91–0.96)[b]

CI: confidence interval; SD: standard deviation.

[a] Data are presented as number (percentage), unless indicated otherwise.
[b] P<0.05.
[c] Data were missing for some previously treated patients (242 foreign-born; 26 born in the Netherlands).
[d] Calculated for patients with acquired drug resistance.

consisted of only one case and were excluded from further analysis. Of the resulting 14,459 isolates, 8,330 (58%) were unique, of which 1,675 (20%) were the first case in a cluster; and 6,129/14,459 (42%) were clustered cases.

The total number of clusters was 1,676 and the median cluster size was 6 (interquartile range: 3–19). Of the 1,676 clusters, 420 (25%) contained at least one case with resistant TB. Epidemiological cluster investigation was performed for 5,594 (91%) of all 6,129 clustered cases: an epidemiological link was confirmed in 1,674 (30%) clustered cases, likely in 923 (17%) clustered cases and could not be determined in 2,997 (54%) clustered cases.

PDR cases (n=1,445) were classified according to a transmission classification model (Figure 3): 129 cases (9%) were definitely infected in the Netherlands, 404 cases (28%) possibly and 912 cases (63%) were infected abroad or before 1993, when DNA fingerprinting was not systematically performed. PDR patients born in the Netherlands more often had clustered isolates than foreign-born PDR patients (132 (54%) vs 469 (39%), p<0.001) and were more likely to have a confirmed epidemiological link with a previous case in the cluster (54 (22%) vs 75 (6%), p<0.001) (data not shown).

Discussion

This study, based on a large number of cases and molecular typing data from the Netherlands, covering many years, revealed that antituberculosis drug resistance has increased since 1993 in patients born in the Netherlands and since 2005 in those foreign-born, and that resistance was more frequent among foreign-born patients. The increasing trend was mainly related to an increase in resistance to isoniazid, the cornerstone of first-line treatment. Furthermore, more than 90% of the drug resistance seen was a result of transmission. Our classification model suggests that transmission of resistant strains occurred in more than 60% of the cases before 1993 or abroad, and in 9% of the cases definitely in the Netherlands. Although ADR was rare, and mainly related to previous treatment abroad, in 45/122 cases it was associated with previous treatment failure in the Netherlands. Patient files should be retrieved and treatment history examined to gain more insight into the possible acquisition of resistance in the Netherlands.

The impact of the unexpected increase in antituberculosis drug resistance among patients born in the Netherlands is likely to be limited, because the majority of TB drug resistance is still mainly found in foreign-born patients, as reported previously [7].

The largest increase has been seen since 2005–06, when resistance among foreign-born patients has also been increasing. We suspect that the increase might be a result of enhanced migration from TB-endemic countries with high rates of TB drug resistance [18]. Concomitant intermingling of people born in the Netherlands and people with different ethnic backgrounds born outside the Netherlands might have resulted in the spread of drug-resistant TB. This might explain the increase in isoniazid resistance, as such

Table 3

Comparison of rates of resistance to various antituberculosis drugs in patients with acquired and primary drug resistance[a] among 1,622 patients diagnosed with drug-resistant tuberculosis, the Netherlands, 1993–2011

Characteristic	Acquired DR n=122	Primary DR n=1,500	Crude OR (95% CI)	Adjusted OR[c] (95% CI)
	n (%)[b]	n (%)[b]		
Drug resistance				
Isoniazid	86 (70)	882 (59)	1.67 (1.12–2.50)[d]	1.87 (0.83–4.18)
Rifampicin	36 (30)	130 (9)	4.41 (2.87–6.76)[d]	3.02 (1.43–6.38)[d]
Streptomycin	72 (59)	953 (64)	0.82 (0.57-1.20)	1.27 (0.67–2.43)
Ethambutol	17 (14)	47 (3)	3.86 (2.07–7.22)[d]	1.40 (0.57–3.47)
Pyrazinamide	8 (7)	53 (4)	1.49 (0.67–3.33)	1.32 (0.54–3.22)
Multidrug resistance (isoniazid and rifampicin)	32 (26)	109 (7)	4.53 (2.90–7.09)[d]	5.43 (3.39–8.65)[d,e]
Other				
Mean age in years (SD)	39 (16)	33 (15)	1.02 (1.01–1.03)[d]	1.03 (1.01–1.05)[d]
Foreign born	106 (87)	1,242 (83)	1.38 (0.80–2.37)	1.68 (0.66–4.30)
Median duration of stay in the Netherlands in years (IQR)[f]	2 (0–11)	4 (1–10)	1.00 (0.98–1.02)	0.99 (0.96–1.03)

CI: confidence interval; DR: drug resistance; IQR: interquartile range; OR: odds ratio; SD: standard deviation; TB: tuberculosis.

[a] Acquired drug resistance refers to resistance in previously treated TB cases; primary drug resistance refers to drug resistance in new TB cases.

[b] Data are presented as number (percentage), unless indicated otherwise.

[c] Adjusted for drug-specific resistance, age and being foreign-born.

[d] P<0.05.

[e] Adjusted for age and being foreign-born.

[f] Calculated for foreign-born patients.

an increase has also been observed in other countries [19]. Furthermore, next to certain 'host-related factors' [20], *M. tuberculosis* in general might have gained a higher ability to withstand treatment, resulting in more persistent infections and higher rates of transmission to other people. Possibly, particular resistance mutations might be less deleterious than others or certain compensatory mutations might make up for any loss of fitness caused by the resistance mutation [21,22]. Borrell et al. demonstrated that the most common isoniazid resistance-conferring mutation in clinical settings reduced isoniazid activation while maintaining virulence in mice [23].

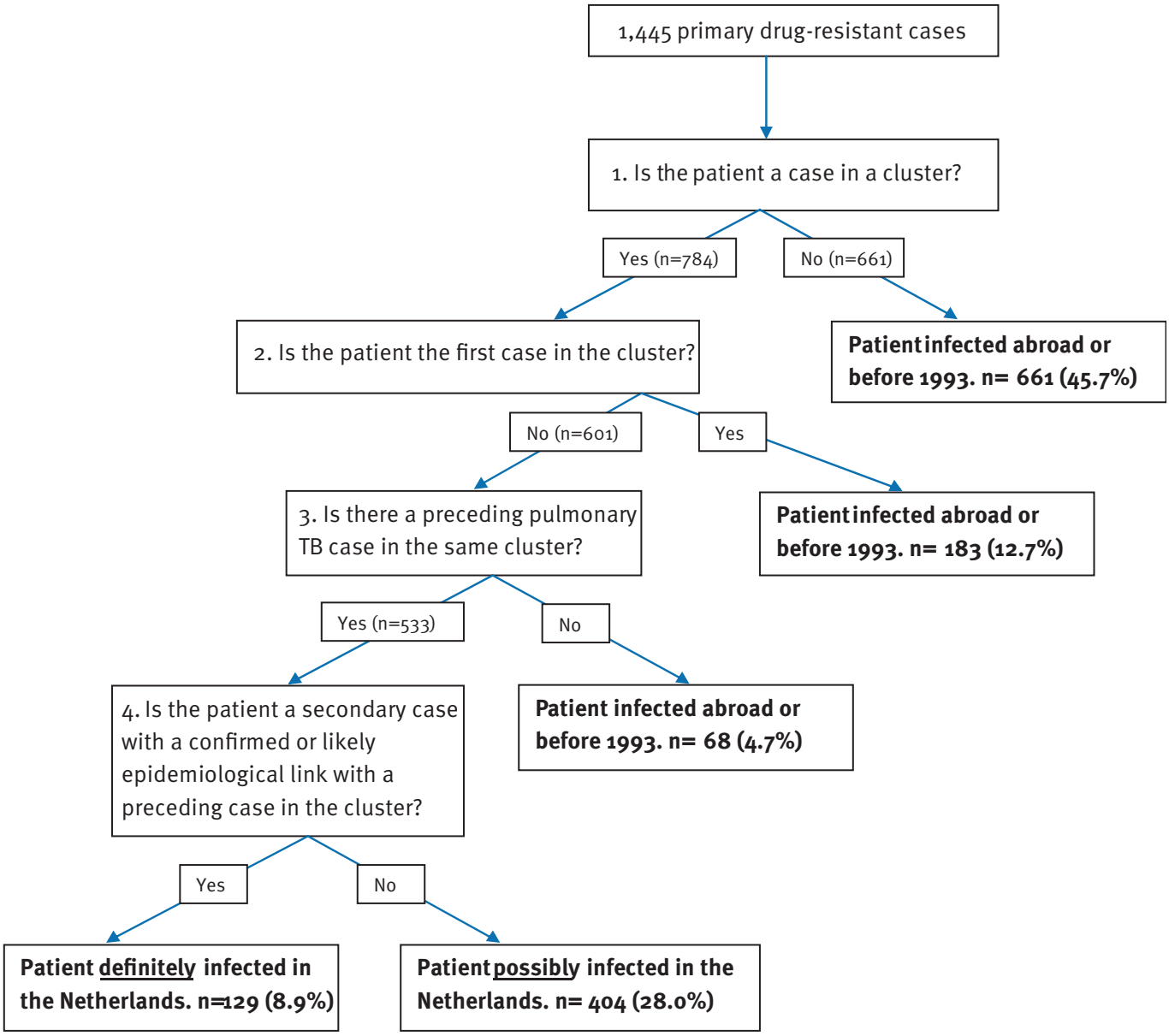
The pronounced increase in resistance to regularly used drugs is considered highly relevant as these are the cornerstone of treatment and may perhaps reflect a trend in other European countries where large numbers of migrants are received [3,24]. Although MDR-TB is diagnosed relatively rarely in the Netherlands [6], we should closely monitor the seemingly increasing MDR-TB trend, as its treatment is costly, complicated and enduring [3]. In Europe, trends in MDR-TB over the past five years have differed substantially by country [3]. The MDR-TB rate remained stable in European Union/European Economic Area (EU/EEA) countries (4.5% in 2011), while in non-EU/EEA countries, it increased from 20.3% in 2007 to 30.2% in 2011.

The 23% drug resistance among previously treated patients found in our study was similar to the median prevalence of resistance in previously treated patients found in a recent global surveillance project (25.1%) [8]. Our finding that resistance in *M. tuberculosis* was mainly the result of transmission was also in line with global findings [25]. In 129 of 784 clustered PDR cases, cluster investigation confirmed transmission of resistance in the Netherlands, despite the measures to prevent TB transmission such as contact tracing and screening of risk groups. Among foreign-born cases, clustering does not always reflect transmission in the Netherlands. In particular, clustered cases originating from the same foreign country might have been infected in their home country by strains of the predominant genotypes in those countries. This could also be an explanation for the low percentage of foreign-born PDR cases with a confirmed epidemiological link. In general, the proportion of patients definitely infected in the Netherlands is presumably underestimated, as transmission in a public space often remains unconfirmed in epidemiological cluster investigations. Furthermore, cluster investigation in foreign-born cases can be hindered by communication difficulties [26].

There are limitations associated with this study. Firstly, drug resistance trends could have been influenced by the change in DST method in 2004. However, the most

Figure 3

Classification model to determine the place of infection for tuberculosis patients with primary drug-resistant strains, the Netherlands, 1993–2011



pronounced increase in resistance was seen in the period since 2005, in which only the MGIT DST method was used. Moreover, DST at the National Reference Laboratory in the Netherlands has always been checked by WHO proficiency testing. Secondly, pyrazinamide susceptibility testing was less reliable before 2009 and may therefore have had an effect on the results. However, due to the rare occurrence of pyrazinamide resistance, this probably had little effect on the overall trends. Thirdly, misclassification of ADR and PDR cases could have occurred because the classification was based on self-reported treatment history. Fourthly, 14% of all patients with drug-resistant TB could not be classified as ADR or PDR at all, because their treatment history was unknown. Besides, ADR cases could have been PDR cases if they were reinfected with a resistant strain that differed from the strain they were previously treated for. For instance, a previous study has shown that reinfection with a different strain occurred in 16% of all patients with Dutch nationality who had been infected before 1981 [27]. Additionally, ADR patients born in the Netherlands whose parents had been born abroad could have acquired resistance in their parents' country of origin, when visiting friends and family. The same may apply to patients who may have worked in a high TB burden country. On the other hand, the 14 foreign-born ADR cases with unknown place of previous treatment could have acquired resistance in the Netherlands. Lastly, for 454 patients who were part of clusters whose data could not be matched to that of

the National Reference Laboratory, the place of transmission could not be determined.

In conclusion, the increase in resistance of *M. tuberculosis* among patients born in the Netherlands and the recent increase among foreign-born patients have not led to an increase in the incidence of TB in the Netherlands, as the incidence has remained stable over the last few years [6]. With a high degree of transmission of resistant strains abroad and a large proportion of *M. tuberculosis* drug resistance among migrants, the problem of resistance in the Netherlands is closely related to the resistance problems in the migrants' countries of origin. This highlights the importance of early detection of TB, resistance screening and treatment programmes, especially in migrants originating from high-endemic countries with a high resistance rate. In these countries, preventive measures such as improved case detection, individualised treatment and improved drug supply and distribution could reduce the risk of acquiring resistance and its subsequent transmission. Our findings may be representative of the situation in other low-endemic European countries with a relatively large proportion of migrants. Generally, only little is known about trends in resistance as testing and reporting is currently not sufficiently frequent or complete in many countries. The capacity to respond adequately to the threat of drug-resistant TB requires more detailed information on the magnitude of this problem. Therefore more research is needed in other European countries. Resistance monitoring and surveillance remain highly important activities.

Acknowledgments

Marianne van der Sande and Wim van der Hoek are acknowledged for their critical suggestions to optimise the manuscript. We thank Hanna Guimaraes, Jessica de Beer and Erika Slump for their technical support and expertise. Additionally, we thank Jan van de Kassteele for his help on the statistical analysis and Henrieke Schimmel for her assistance on data providing. We also thank the staff of the RIVM tuberculosis laboratory for their practical work regarding the RFLP and VNTR typing of *M. tuberculosis* isolates, the Municipal Health Services for their collaboration in the nationwide tuberculosis surveillance and the microbiological laboratories for supplying the *M. tuberculosis* isolates to the RIVM.

Conflict of interest

None declared.

Authors' contributions

CR participated in the coordination of the study, performed the statistical analyses and had the lead in drafting the manuscript. AvG participated in the design of the study, in performing the statistical analyses and drafting the manuscript. GdV was involved in developing the transmission classification model and in drafting the manuscript. CE participated in the design of the study and drafting the manuscript. JvR participated in the coordination of the study. HKA participated in performing the statistical analyses and drafting the manuscript. HdN contributed to the drafting of the manuscript. MK was involved in the design of the study and was responsible for the RFLP and VNTR typing of the isolates. DvS participated in the design and coordination of the study and was responsible for the RFLP and VNTR typing of the isolates and participated in drafting the manuscript. All authors read and approved the final manuscript.

References

1. World Health Organization (WHO). Global tuberculosis report 2013. Geneva: WHO; 2013. Available from: http://www.who.int/tb/publications/global_report/en
2. Nachega JB, Chaisson RE. Tuberculosis drug resistance: a global threat. Clin Infect Dis. 2003;36(Suppl 1):S24-30. http://dx.doi.org/10.1086/344657
3. European Centre for Disease Prevention and Control (ECDC)/ World Health Organization Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2013. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/Tuberculosis-surveillance-monitoring-2013.pdf
4. World Health Organization (WHO). The Stop TB Strategy. Building on and enhancing DOTS to meet the TB-related Millennium Development Goals, 2006. Geneva: WHO; 2006. Available from: http://www.who.int/tb/publications/2006/who_htm_tb_2006_368.pdf?ua=1
5. World Health Organization (WHO). Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva: WHO; 2010. Available from: http://whqlibdoc.who.int/publications/2010/9789241599191_eng.pdf
6. Slump E, Erkens CG, van Hunen R, van Rest JF, Schimmel HJ, van Soolingen D. KNCV Tuberculosis Foundation. 'Tuberculose in Nederland 2011', Surveillancerapport over de tuberculosesituatie in Nederland. [Tuberculosis in the Netherlands, Surveillance report]. The Hague: KNVC Tuberculosis Foundation; 2012. Dutch. Available from: http://www.rivm.nl/dsresource?objectid=rivmp:210602&type=org&disposition=inline&ns_nc=1
7. Lambregts-van Weezenbeek CS, Jansen HM, Veen J, Nagelkerke NJ, Sebek MM, Van Soolingen D. Origin and management of primary and acquired drug-resistant tuberculosis in The Netherlands: the truth behind the rates. Int J Tuberc Lung Dis. 1998;2(4):296-302.
8. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K, et al. Epidemiology of antituberculosis drug resistance 2002-07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Lancet. 2009;373(9678):1861-73. http://dx.doi.org/10.1016/S0140-6736(09)60331-7
9. Dutch Foundation of the Working Party on Antibiotic Policy (SWAB). Nethmap 2012. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. Nijmegen/ Bilthoven: SWAB/ Centre for Infectious disease control (CIb) of the National Institute for Public Health and the Environment of the Netherlands (RIVM); 2012. Available from: http://www.swab.nl/swab/cms3.nsf/uploads/E8426668DC9BC944C1257A24006252DE/$FILE/Nethmap_Maran_2012.pdf
10. Van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol. 1993;31(2):406-9.
11. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol. 2006;44(12):4498-510. http://dx.doi.org/10.1128/JCM.01392-06
12. van Klingeren B, Dessens-Kroon M, van der Laan T, Kremer K, van Soolingen D. Drug susceptibility testing of Mycobacterium tuberculosis complex by use of a high-throughput, reproducible, absolute concentration method. J Clin Microbiol. 2007;45(8):2662-8. http://dx.doi.org/10.1128/JCM.00244-07
13. Palomino JC, Traore H, Fissette K, Portaels F. Evaluation of Mycobacteria Growth Indicator Tube (MGIT) for drug susceptibility testing of Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 1999;3(4):344-8.
14. van Soolingen D, de Haas PE, Hermans PW, Groenen PM, van Embden JD. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. J Clin Microbiol. 1993;31(8):1987-95.

15. de Beer JL, van Ingen J, de Vries G, Erkens C, Sebek M, Mulder A et al. Comparative study of IS6110 restriction fragment length polymorphism and variable-number tandem repeat typing of Mycobacterium tuberculosis Isolates in the Netherlands, based on a 5-year nationwide survey. J Clin Microbiol. 2013;51(4):1193-8. http://dx.doi.org/10.1128/JCM.03061-12
16. de Vries G, Baars HW, Sebek MM, van Hest NA, Richardus JH. Transmission classification model to determine place and time of infection of tuberculosis cases in an Urban Area. J Clin Microbiol. 2008; 46(12):3924-30. http://dx.doi.org/10.1128/JCM.00793-08
17. Lambregts-van Weezenbeek CS, Sebek MM, van Gerven PJ, de Vries G, Verver S, Kalisvaart NA, et al. Tuberculosis contact investigation and DNA fingerprint surveillance in The Netherlands: 6 years' experience with nation-wide cluster feedback and cluster monitoring. Int J Tuberc Lung Dis. 2003;7(12 Suppl 3):S463-70.
18. Central Bureau of Statistics (CBS). StatLine. The Hague: CBS. [Accessed 19 Mar 2014]. Available from: http://statline.cbs.nl/statweb/?LA=en
19. Jenkins HE, Zignol M, Cohen T. Quantifying the burden and trends of isoniazid resistant tuberculosis, 1994-2009. PLoS One. 2011;6(7):e22927. http://dx.doi.org/10.1371/journal.pone.0022927
20. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax. 2006;61(2):158-63. http://dx.doi.org/10.1136/thx.2005.045963
21. Comas I, Borrell S, Roetzer A, Rose G, Malla B, Kato-Maeda M, et al. Whole-genome sequencing of rifampicin-resistant Mycobacterium tuberculosis strains identifies compensatory mutations in RNA polymerase genes. Nat Genet. 2011;44(1):106-10. http://dx.doi.org/10.1038/ng.1038
22. van Soolingen D, de Haas PE, van Doorn HR, Kuijper E, Rinder H, Borgdorff MW. Mutations at amino acid position 315 of the katG gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of Mycobacterium tuberculosis in the Netherlands. J Infect Dis. 2000;182(6):1788-90. http://dx.doi.org/10.1086/317598
23. Borrell S, Gagneux S. Infectiousness, reproductive fitness and evolution of drug-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13(12):1456-66.
24. Espinal MA, Laszlo AP, Simonsen L, Boulahbal F, Kim SJ, Reniero A, et al. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. N Engl J Med. 2001;344(17):1294-303. http://dx.doi.org/10.1056/NEJM200104263441706
25. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet. 2010; 375(9728):1830-43. http://dx.doi.org/10.1016/S0140-6736(10)60410-2
26. Mulder C, van Deutekom H, Huisman EM, Meijer-Veldman W, Erkens CG, van Rest J, et al. Coverage and yield of tuberculosis contact investigations in the Netherlands. Int J Tuberc Lung Dis. 2011;15(12):1630-7. http://dx.doi.org/10.5588/ijtld.11.0027
27. de Boer AS, Borgdorff MW, Vynnycky E, Sebek MM, van Soolingen D. Exogenous re-infection as a cause of recurrent tuberculosis in a low-incidence area. Int J Tuberc Lung Dis. 2003;7(2):145-52.